Role of Non-feminizing Estrogens in Brain Protection from Cerebral Ischemia and Alzheimer’s Disease Neuropathology

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Estrogens are potent and efficacious neuroprotectants both in vitro and in vivo in a variety of models of neurotoxicity. We determined the structural requirements for neuroprotection in an in vitro assay using a panel of more than 100 novel estratrienes, synthesized to reduce or eliminate estrogen receptor (ER) binding. We observed that neuroprotection could be enhanced by as much as 200-fold through modifications that positioned a large bulky group at the C2 or C4 position of the phenolic A ring of the estratriene. Further, substitutions on the B, C or D rings either reduced or did not markedly change neuroprotection. Collectively, there was a negative correlation between binding to ERs and neuroprotection with the more potent compounds showing no ER binding. In an in vivo model for neuroprotection, transient cerebral ischemia, efficacious compounds were active in protection of brain tissue from this pro-oxidant insult. Finally, estradiol protected brains from insult-induced Alzheimer’s disease (AD) neuropathology, including, activation of apoptosis, stimulation of APP production, hyperphosphorylation of tau, activation of cyclin-dependent kinases, and activation of catastrophic attempts at neuronal mitosis. Collectively, these results demonstrate that non-feminizing estrogens are neuroprotective and protect brain from the induction of AD-like neuropathology in an animal model. These features of non-feminizing estrogens make them attractive compounds for assessment of efficacy in AD and stroke, as they are not expected to show the side effects of chronic estrogen therapy that are mediated by ER-mediated actions in the liver, uterus and breast. (This work was supported by the National Institutes of Health (P20 GM109098, P01 AG022550, P01 AG027956, U54 GM109492).
"Cerebrovascular aging: novel mechanisms and consequences"
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The brain has high energy requirements and very little energy reserves. The human brain receives almost 15 percent of the cardiac output through a network of over 600 km of capillaries. In the brain the number of endothelial cells is very similar to that of neurons and nearly every neuron is supplied by its own capillary. It is becoming evident that age-related microvascular impairment plays a critical role in the pathogenesis of age-related cognitive decline. The potential roles of microvascular mechanisms involved in cognitive impairment, including microvascular rarefaction and disruption of the blood-brain barrier will be discussed. Also, moment-to-moment adjustment of cerebral blood flow via neurovascular coupling is essential for the maintenance of normal neuronal function. Increased oxidative stress that occurs with aging was shown to impair neurovascular coupling, which likely contributes to a significant age-related decline in higher cortical function, increasing the risk for vascular cognitive impairment. There are suggestions in the literature and new data will be presented that in aged laboratory rodents neurovascular coupling and endothelium-dependent cerebromicrovascular dilation can be rescued, which represents a potential therapeutic target for the promotion of healthy brain aging.
Veronica Galvan  
TOR drives neuronal and brain vascular dysfunction in mouse models of Alzheimer’s disease

We recently showed that chronic treatment with the target-of-rapamycin (TOR) inhibitor rapamycin, a drug that extends lifespan and delays aging in mice, halted and even reversed Alzheimer’s (AD)-like memory deficits, decreased Aβ, and restored cerebral blood flow (CBF) in brains of hAPP(J20) and Tg2576 mice modeling the disease. Reducing TOR activity also restored cognitive function and CBF in mice modeling atherosclerosis, as well as in 36 month-old rats. In very old rats, attenuation of TOR activity was associated with the recovery of cortical network activation and functional hyperemia evoked by somatosensory stimulation. Our data indicate that the mechanisms by which TOR attenuation restores CBF, neuronal activity, and cognitive function may be common to different models of age-associated neurological disease and to brain aging, and single out (a) vascular NO release, and (b) synaptic bouton remodeling as key mechanisms by which TOR attenuation blocks AD-like progression in mice.

To delineate the mechanisms by which TOR regulates synaptic remodeling during aging and in AD we used rapamycin in very old rats, and advanced tissue-specific genetic tools to reduce TOR complex 1 assembly specifically in neurons of adult mice. Moderate, but not drastic reduction of TORC1 assembly in neurons, to levels similar to those achieved by rapamycin treatment, promoted synaptic remodeling and increased autophagy, potentially increasing synaptic vesicle recycling. This was associated with enhanced memory and increased brain glucose uptake, suggestive of increased brain glucose metabolism. We propose that attenuation of TOR in (a) brain vascular endothelial cells and in (b) Aβ-producing parenchymal neurons by pharmacological or genetic means act synergistically to slow the progression of AD dysfunction through the restoration of (1) NO-dependent vasodilation and CBF, increasing vascular Aβ clearance, and (2) by increasing autophagy at synapses, leading to synaptic bouton remodeling, decreased Aβ release, and restored neuronal function in AD. Relieving TOR-dependent inhibition of synaptic remodeling and neurovascular NO release may thus be critical synergistic mechanisms by which rapamycin or genetic TORC1 knockdown preserve network activation, functional hyperemia and cognitive function during aging and in models of AD and other dementias. TOR inhibition may have promise as therapy for AD and potentially other neurodegenerations.
Aging is the primary risk factor for numerous diseases including neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease. One aspect of aging that contributes to the loss of resiliency in the brain that contributes to this increased susceptibility to disease is polarization of microglia to a more pro-inflammatory state and a blunted ability to up-regulate anti-inflammatory factors such as IL-4 and GNDF. We have been investigating approaches that can modulate microglial polarization as potential modifiers of neurodegenerative diseases. One approach has been to use fractalkine, a chemokine that is produced by neurons and the fractalkine receptor is found on microglia. The normal function of fractalkine is to suppress the production of IL1β and TNFα. Loss of the fractalkine receptor produces We have demonstrated that fractalkine is neuroprotective in three different models of Parkinson’s disease, 6-hydroxy dopamine, MPTP and α-synuclein induced dopamine cell death. We are also examining several natural products ability to suppress microglial activation and promote neural repair including astaxanthin which is a carotenoid. We demonstrate that astaxanthin prevents damage from MPTP. A second aspect of increased inflammation in the aged brain is an impact on the neurogenic niche and on the ability of exogenous stem cells to survive and contribute to recovery after brain injury.
The need for sophisticated translational models of aging is growing as the human population is graying and healthcare costs are rising. Nonhuman primates (NHPs) provide an excellent opportunity to study a closely related species that experiences similar aging processes and is relevant for the study of interventions. NHPs have similar morphology, physiology, behavior, and aging processes as humans. Rhesus monkeys share >92% homology and are the most commonly studied NHP, but their size, expense, and a lifespan reaching 40 years, suggests the need for a shorter-lived, smaller NHP for longevity studies. Due to its small size, low zoonotic risk, reproductive efficiency, and relatively low cost to maintain, marmosets are rapidly becoming the preferred NHP for biomedical testing. Both species experience age-related pathology similar to humans, such as cancer, diabetes, arthritis, cardiovascular disease, and neurological decline, and they are paving the way to improving human health and a better understanding of the mechanisms of aging.
Mycobacterial heat shock protein 65 (mbHSP65)-induced atherosclerosis - Preventive oral tolerization and definition of atheroprotective and atherogenic mbHSP65-peptides

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Abstract

Aims Atherosclerosis is a paradigmatic age-related disease that starts early but becomes clinically manifest later in life. Heat shock proteins (HSPs) are important antigens and autoantigens triggering innate and adaptive immune responses initiating the earliest still reversible inflammatory stages of atherosclerosis. We examined the immunological effect of mycobacterial HSP65 (mbHSP65) immunizations, oral mbHSP65-tolerization, and specific atheroprotective and atherogenic reactive T-cell mbHSP65-peptides, in C57BL/6J and ApoE−/− mice.

Methods and Results Mice received 4 mbHSP65 immunizations and/or 8 oral mbHSP65 feedings. Concomitantly with the first immunization/tolerization, mice were either kept on chow diet or high cholesterol diet. The aorta was analysed en face, immunohistologically, and for cytokine production, lymph node and spleen cells were examined for mbHSP65-specific T regulatory cells (Treg) and T-cell reactive mbHSP65-peptides, plasma/sera for lipids, cytokines, and anti-HSPs autoantibodies. We found significantly increased aortal lesion formation after mbHSP65 immunizations and abrogated lesion formation after oral mbHSP65 tolerization even in mbHSP65-immunized mice. The decreased lesion size was accompanied by decreased lipid levels, increased numbers and suppressive capacity of specific and non-specific Tregs, decreased amounts of proinflammatory- and increased amounts of anti-inflammatory cytokines in the aortae, intralesional cells, and circulation. Increased levels of anti-mbHSP65 and anti-mouse-HSP60 antibodies were found after mbHSP65 immunization/tolerization indicating specific (cross)-reactive immunity. Importantly, this study also provides functional proof for the presence of specific T-cell reactive mbHSP65-peptides in early stages of atherosclerosis, and after immunizations with these peptides, we could define their atheroprotective/atherogenic properties.

Conclusions Our results demonstrate that oral mbHSP65-tolerization reduces the inflammatory process associated with mbHSP65-induced atherosclerosis and provides new immunological approaches for the treatment of atherosclerosis with specific atherogenic mbHSP65-peptides.
Pleiotropic response to methionine restriction

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Abstract

Methionine restriction (MR) extends lifespan across different species. The main responses of rodent models to MR are well-documented in adipose tissue (AT) and liver, in which it has been shown to reduce mass and improve insulin sensitivity, respectively. Recently, molecular mechanisms that improved healthspan have been identified in both organs during MR. MR induced a futile lipid cycle concomitant with beige AT accumulation producing elevated energy expenditure. In MR liver, upregulated fibroblast growth factor 21 improved glucose metabolism in a high-fat diet or in aged mice. MR also reduces mitochondrial oxidative stress in various organs such as liver, heart, kidneys, and brain.

The beneficial effects of MR have also been documented in a number of invertebrate model organisms, including yeast, nematodes, and fruit flies. MR not only promotes extended longevity in these organisms, but in the case of yeast has also been shown to improve stress tolerance. In addition, expression analyses of methionine-restricted yeast and Drosophila have identified multiple candidate mediators of the beneficial effects of MR in these models.

Other effects of MR in such areas as cardiac function in response to hyperhomocysteinemia, identification of molecular mechanisms in bone development, and enhanced epithelial tight junction have also been reported. Moreover, rodent models of cancer responded positively to MR as has been shown in colon, prostate, and breast cancer studies. On-going studies should be directed in identifying novel molecular mechanisms of MR that promote healthy aging.
Improving Neuronal and Behavioral Function in Aging with Nutrition


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Research has demonstrated, in both human and animals, that cognitive functioning decreases with age, to include deficits in processing speed, executive function, memory, and spatial learning. The cause of these functional declines is not entirely understood; however, neuronal losses and the associated changes in the activity of neurotransmitters, secondary messengers, and their receptors may be caused by long term increases in and susceptibility to oxidative stress and inflammation. Therefore, one approach to improving neuronal functioning might be to alter the neuronal environment to reduce the impact of oxidative and inflammatory stressors. Research conducted in our laboratory, initially with animals but more recently with humans, has shown that consumption of berry fruits, i.e., strawberries and blueberries which are high in polyphenolics, can prevent and even reverse age-related cognitive and neuronal deficits. The polyphenolic compounds found in these foods may exert their beneficial effects indirectly, through their ability to lower oxidative stress and inflammation, or directly, by altering neuronal structure and signaling involved in neuronal communication. Additionally, the polyphenolics in different berry fruits may have differential effects on cognition and inflammation/oxidative stress. Therefore, dietary interventions with polyphenolic-rich foods may be one strategy to forestall or even reverse age-related neuronal deficits.
Brain aging has long been associated with motor and cognitive dysfunction along with a progressive increase in oxidative burden. Popular interventions such as antioxidant intake or moderate exercise are often recommended to attain healthy brain aging and reduce oxidative stress. Furthermore, these two interventions are often coupled together in anticipation of additive effects based on the rationale that each intervention alone activates anti-aging mechanisms ameliorating brain function. However, the nature of the interaction between exercise and antioxidants may not be as simple as first thought with evidence indicating that in some instances antioxidants can antagonize the beneficial effects of moderate exercise. Our laboratory has set out to study the nature of the interaction between exercise and antioxidants in the context of ApoE genotype, aging or sex using a comprehensive behavioral profiling to paint an accurate and specific picture of the outcomes of the interventions. In separate studies, male and female mice expressing the human ApoE3 or ApoE4 genes or C57BL/6 mice of different ages were assigned to one of the following experimental groups: Sedentary- Control Diet; Sedentary- Aox Diet; Exercised- Control Diet; Exercised- Aox Diet. The Aox Diet was supplemented with 1.65 mg ascorbate /g diet and 0.825 mg α-tocopheryl acetate/ g diet and fed ad libitum. The exercised mice received a forced exercise regimen training of 1h using an inclined treadmill. The respective treatments were followed for 8 weeks pre-treatment period and throughout behavioral testing, which measured spontaneous activity, musculoskeletal reflexes, strength, balance, coordination, spatial learning and memory, anxiety, learning and cognitive flexibility. Our data indicated that ApoE genotype, age and sex were important factors to consider and led to differential outcomes of the interventions. Overall, our studies did not reveal any major additive or antagonistic effect of moderate exercise and antioxidant intake, supporting that each intervention most likely improves brain function via independent pathways.
Is Pregnancy an Appropriate Time to Intervene to Improve Long-term Offspring Health?

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Caloric restriction has shown itself to be the most reproducible and promising intervention to improve health outcomes in laboratory animals. An intense and expanding area of research is focused on discovering easily achievable interventions that can have long-lasting positive effects. Although exercise is widely recognized as an important part of a healthy lifestyle and is known to improve cardiovascular and metabolic health, seemingly few people have the time or motivation for physical activity. If exercise during pregnancy could not only protect individuals against disease, but also provide lifelong health benefits to their developing child, there should be more incentive to exercise. As part of this study, we hypothesized that maternal exercise during pregnancy and nursing will improve offspring insulin sensitivity and protect them against chemical carcinogens. Female mice were given free access to running wheels in their home cages prior to and during pregnancy and nursing and long-term health outcomes were measured in offspring. Both male and female offspring born to exercised dams had significantly enhanced insulin sensitivity during adulthood compared to offspring born to sedentary moms. Further, both male and female offspring had significantly decreased tumor incidence and tumor number as a result of maternal exercise. Future studies will explore the mechanisms behind these protective effects. While our studies have been completed in rodents, we are currently working on projects that extend our work to humans. Utilizing neonatal foreskin tissue, which is readily available following circumcision, we have shown that dermal primary fibroblasts can be isolated and grown in culture for living functional studies to examine developmental programming in humans. Our findings highlight pregnancy as a sensitive period when positive lifestyle interventions could have significant and long-lasting beneficial effects on offspring metabolism and disease risk.
The incidence of many cancers increases exponentially with age and age is the biggest single risk factor for most cancers. The reasons for this are not well understood. Presumably, longevity and suppression of disease, including cancer, depends on long-lived cells, such as terminally differentiated neurons, being able to harness the dynamic epigenome so as to be able to maintain a stable phenotype. The presumptive mechanisms that act to maintain chromatin homeostasis over the lifecourse can be termed “chromostasis”. The epigenome can also be exploited in cancer therapies and, in the long term, to promote healthy aging and suppression of age-associated diseases, such as cancer.
Genome instability: a conserved mechanism of aging?

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Genome instability has been implicated as a main causal factor in age-related cellular degeneration and death since the 1950s when the first evidence emerged that low doses of ionizing radiation can accelerate aging. Genome instability as a driver of aging is an attractive hypothesis for two main reasons. First, since DNA is the primary informational macromolecule of the genome, loss or alteration of its sequence is essentially irreversible, which is generally not true for changes in other macromolecules, such as proteins. Second, heritable mutations in multiple genes involved in genome maintenance in both humans and mice have been found associated with segmental progeria; there is little evidence that the same is true for other gene families thought to be involved in longevity, such as antioxidant defense and autophagy. However, due to the random nature of genome instability, alterations in individual cells are obscured when analyzing bulk cells or tissues. This means that we do not know the severity of the genomic mutation load of aging cells, which has essentially constrained the establishment of reliable cause and effect relationships. I will describe several approaches to comprehensively characterize the landscape of somatic genome alterations in cells and tissues of aging animals and discuss their possible involvement in age-related functional decline and disease.
Are stem cell therapies a realistic option for diseases of the aged brain?
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We are currently in an interesting phase in the development of stem cell therapies for diseases of the aged brain. It is now almost 25 years since the first clinical trials of cellular therapeutics in Parkinson’s disease. But still there is no licensed cellular therapy for any CNS disorder under any jurisdiction. Perhaps coincidentally, there has never been a time during this period when one group of scientists has not been applauding an imminent breakthrough, while another has been warning against charlatanism, over-zealous commercialization, or plain bad science.

How in this context should we consider the two recent reports of clinical success in trials of stem cells for stroke, or the current European initiative to reinvigorate fetal transplantation in Parkinson’s Disease? More fundamentally, how are prospects altered by the emergence of human pluripotent stem cells, or indeed the now viable approach of ‘direct reprogramming’?

In this talk, I’ll try to clarify where we are now in regards to cellular therapies for the aged brain, what new possibilities are emerging, and why a true regenerative medicine of the brain is probably still a good way off.
Stem cells reside primarily in two restricted zones in the adult rodent brain and support continued neurogenesis throughout adulthood. Recently, we reported that in addition to promoting neurogenesis in the subventricular zone (SVZ), stem cells also repair the adjacent epithelial lining of the lateral wall of the lateral ventricles, the ependyma. New ependymal-like cells are generated on an ‘as needed’ basis and ependyma integrity along the lateral wall in mice appears to be maintained through aging. This ability of the adult brain to generate new cells raised the exciting possibility that adult stem cells may be used for repair following brain injuries or disease in humans. However, SVZ functions in humans are greatly diminished by 2 years and humans typically show ependymal cell denudation, accompanying periventricular gliosis and ventriculomegaly with increased age. While the demise of the ependymal layer has been inextricably linked to hydrocephaly/ventriculomegaly, and many neurological/psychiatric illnesses (e.g., autism, ADHD, schizophrenia) display ventriculomegaly, the molecular and cellular mechanisms underlying ependyma loss and its involvement in disease mechanisms (initiation or progression) remain largely enigmatic. Our studies address the striking differences in the SVZ niche between humans and mice, and highlight the adaptive (maladaptive) significance of these differences to ependyma health and longevity. Specifically in both mouse and human, we characterize the adult SVZ stem cell niche in aging; the impact injury and disease have on stem cell niche dynamics and function; the consequence of stem cell loss; and the importance of ependymal lining integrity through aging. Our studies reveal that mouse brain stem cells function in neurogenesis and regenerative repair of the ependymal lining of the lateral ventricles, functions that continue into old age. In contrast, humans do not appear to maintain a robust stem cell niche, have greatly diminished neurogenesis and typically show periventricular gliosis and ventriculomegaly. The importance of the brain’s stem cell-mediated repair mechanisms are addressed by the development of novel mouse models that mimic the adult and aging human brain.
Stem cell therapies in preclinical models of stroke. Is the aged brain microenvironment refractory to cell therapy?

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ABSTRACT

Attractive therapeutic strategies to enhance post-stroke recovery of aged brains include methods of cellular therapy that can enhance the endogenous restorative mechanisms of the injured brain. The translational failure of experimental therapies in aged subjects might at least partially be related to the aged brain microenvironment. However, in previous work we have shown that G-CSF alone is effective in improving behavioral recovery after stroke in aged rats. Here, we tested the hypothesis that treating post-stroke aged rats with the combination of bone marrow-derived mononuclear cells (BM MNC) or bone marrow-derived mesenchymal cells BM MSC and G-CSF might improve the long term (56 days) functional outcome.

To this end, $1 \times 10^6$ syngeneic BM MSC and BM MNC per kg bodyweight (BW) in combination with G-CSF (50µg/kg, continued for 28 days) were administered via the jugular vein to Sprague-Dawley rats six hours post-stroke. Infarct volume was measured by magnetic resonance imaging 3 and 48 days post-stroke and additionally by immunohistochemistry at day 56. Functional recovery was tested during the entire recovery period. Daily G-CSF treatment led to robust and consistent improvement of neurological function, but did not
alter final infarct volumes. The combination of G-CSF and BM MNC, did not further improve post-stroke recovery. The lack of an additional benefit may be due to an hitherto not well investigated interaction between both approaches and, to a minor extent, to the insensitivity of the aged brains to regenerative mechanisms. Also considering recent findings on other tandem approaches involving G-CSF in animal models featuring relevant co-morbidities, we conclude that such combination therapies are not the optimal approach to treat the acutely injured aged brain.
There is at present a huge disconnect between levels of funding for basic research on fundamental mechanisms of biological aging and, given demographic projections, the anticipated enormous social and economic impacts of a litany of chronic diseases for which aging is by far the major risk factor: Dementias of the Alzheimer Type (several distinct pathogenetic forms?), Parkinson’s Disease/Lewy Body Dementia, Frontotemporal Dementia, Multi-Infarct Dementia, Age-Related Macular Degeneration, Ocular Cataracts, Presbycusis, Arteriosclerosis (several forms), Ischemic & Non-Ischemic Heart Disease, Chronic Pulmonary Obstructive Disease, Chronic Renal Disease, Benign Prostatic Hyperplasia, Sarcopenia, Peripheral Neuropathies, Osteoporosis, Osteoarthritis, Benign & Malignant Neoplasms, etc.). One valuable approach, recently instigated by Felipe Sierra & colleagues at the US National Institute on Aging, is the development of a Geroscience Interest Group among essentially all of the NIH institutes. Another approach is to seek major escalations of private funding. The billionaire’s club is a cogent potential example (http://www.forbes.com/billionaires/). We will need to give their members a list of our research priorities. While I will present my current personal priorities (a moving target), my goal is to encourage brisk discussions among all of you throughout this meeting and, perhaps, to emerge with a consensus for the top five or six—e.g.: Model Organisms: Much more emphasis upon Healthspan Research & a Search for Models of Human Dementias; Comparative Geroscience: Phylogenetically Closely Related Organisms with Contrasting Healthspans; Human Genetics & Somatic Cell Genetics: Antigeroid Alleles, including Suppressor Alleles; Epigenetics: Modulations of Epigenetic Drift; Environment: Epidemiological Research Relevant to Early Human Development & Transgenerational Inheritance; Translational Research: Pre-Clinical Studies in Pet Dogs (e.g., Rapalogs).
Davide De Francesco

Background: Despite successful combination anti-retroviral therapy (cART), HIV-positive individuals have a shorter expected life span compared to HIV-negative individuals due to an increased risk of non-AIDS comorbidity, the risk of which increases with natural aging. This has led to the hypothesis that HIV-positive persons might suffer from accentuated or accelerated aging. The ComorBidity in Relation to AIDS (COBRA) project was established to evaluate the link between HIV infection and age-associated comorbidities. The aim of this study is to compare well-known biomarkers of aging in HIV-positive and appropriately chosen and comparable HIV-negative controls and identify factors associated with advanced biological age.

Methods: Participants were recruited from two study sites, Amsterdam and London, as part of the COBRA project. HIV-positive individuals on cART and with long-term viral suppression were recruited at HIV outpatient clinics. HIV-negative controls, comparable to the HIV-positive group on socio-demographics and life-style factors, were recruited from sexual health clinics affiliated with the HIV clinics. A combination of a set of 10 biomarkers of age (the MARK-AGE algorithm; Bürkle et al., manuscript in preparation) was used to assess the biological age of individuals. Univariate and multivariate statistical methods were used to compare the difference between predicted biological age and chronological age across groups and evaluate its association with life-style factors, markers of HIV infection and clinical events.

Results: 133 HIV-positive individuals [93% male, median age (IQR): 55 (51, 62) years] and 79 comparable HIV-negative individuals [92% male, median age (IQR): 57 (52, 64) years] over 45 years were recruited. The mean (95% CI) difference between biological and chronological age was significantly greater than zero for both HIV-positive and HIV-negative individuals [13.3 years (11.6, 14.9), p<0.01 and 6.0 years (4.2, 7.8), p<0.01, respectively]. The difference was also significantly greater in HIV-positive individuals compared to controls (p<0.01). In HIV-positive individuals, a positive, although only weak, correlation was found with duration of infection (r=0.18, p=0.04) and cART (r=0.17, p=0.05) and time with a CD4+ T cell count below 200 cells/µL (r=0.18, p=0.04). Individuals chronically co-infected with Hepatitis B virus also showed a greater difference between biological and chronological age compared to non-co-infected HIV-positive individuals [mean (95% CI): 22.0 years (13.8, 30.1) vs. 12.9 years (10.9, 14.8), p=0.01].

Conclusion: These results support the hypothesis that chronic HIV infection, despite successful treatment, is accompanied by a tangible age advancement. As the biological age estimation included markers measured in blood lymphocytes, its significant increase, compared to chronological age as well as to controls, in HIV-positive individuals may be partly reflective of the effects of HIV on the lymphocyte compartment. Moreover, unmeasured confounding may also be responsible for the increased biological age observed in HIV-positive individuals. Longitudinal studies are needed in order to further elucidate whether there is a causal link between HIV and advanced aging, and whether this represents accentuated or accelerated aging.
**Brain MRI changes associated with poorer cognitive function despite suppressive antiretroviral therapy**

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**Background.** Grey and white matter (GM and WM) abnormalities have been reported in HIV-positive subjects. However, data are sparse in virologically suppressed cohorts compared with appropriate control populations.

**Methods.** We assessed cognitive function across 6 domains, brain tissue volumes (using T1 structural MRI) and WM microstructure (using diffusion weighted MRI) in 134 HIV-positive individuals on suppressive combination antiretroviral therapy (median [interquartile range] 56 [45-82] years) and 79 demographically comparable HIV-negative subjects (57 [47-80] years). To delineate localized differences in brain structure between HIV-positive subjects and controls, whole brain (voxelwise) analysis of GM volume (GMV), WM volume (WMV), fractional anisotropy (FA), mean, axial and radial diffusivity (MD, AD and RD respectively) were performed using permutation testing, adjusting for age, intracranial volume and scanner type. To relate imaging findings to cognition, measures of GMV, FA and cognitive domain T-scores were integrated using the k-means clustering statistical method.

**Results.** HIV-positive subjects had significantly lower T-scores, representing poorer cognitive function, in 4/6 cognitive domains (median T-scores were: attention, 50.2 vs. 56.8; executive function, 49.3 vs. 52.3; motor function, 48.5 vs. 51.2; processing speed, 51.2 vs. 54.6 (p<0.01 for all) in HIV-positive subjects vs. controls respectively). Voxelwise analyses demonstrated significantly lower GMV, principally in the intra- and supracalcarine cortices and the lingual gyrus (35%, 27% and 13% voxels affected), in HIV-positive subjects vs. controls. Despite the absence of WM atrophy, widespread abnormalities in WM microstructure (reduced FA with increased MD and RD) were evident in the HIV-positive group and were more severe in those with prior AIDS. K-means clustering analysis optimally clustered subjects into a higher GMV/FA cluster (n=99) and a lower GMV/FA cluster (n=109). HIV-positive subjects were more likely to be members of the lower cluster (odds ratio, 2.74 (95% confidence interval 1.53-4.98), p<0.001). This cluster also exhibited poorer cognitive function (figure 1).

**Conclusion.** Cognitive impairment, GM atrophy and WM microstructural abnormalities were evident in HIV-positive individuals despite fully suppressive antiretroviral therapy. Future work to understand the underlying pathogenesis of these imaging changes is warranted.
Figure 1. Jitterplot of cognitive domain T-scores grouped by k-means cluster analysis
Contributors to immune senescence during treated HIV-1 infection.

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Objectives: HIV-positive individuals on successful cART are reported to have higher rates of age-associated co-morbidities than the general population. We analysed cART-treated HIV-positive individuals (HIV+) aged >45 years for characteristics of increased immunological ageing, and compared the findings to those from two groups of uninfected controls.

Methods: We determined CD4⁺ and CD8⁺ counts, T-cell activation, exhaustion and senescence levels in HIV-positive individuals on cART with undetectable viraemia (<50 copies/mL for >12 months) over 45 years of age (HIV+) and HIV-negative participants with comparable age, ethnicity and risk-behaviour (HIV-) in the COBRA (Co-morBidity in Relation to Aids) cohort study. Age-matched blood bank donors (BBD) were analysed for comparison.

Results: HIV+ persons on cART exhibited incomplete CD4⁺ T-cell restoration and elevated CD8⁺ T-cell counts compared to HIV- COBRA participants. Within the CD4⁺ compartment, T-cell activation (CD4⁺CD38⁺HLA-DR⁺) and exhaustion (PD-1⁺) was greater in the HIV+ group when compared to HIV- COBRA. Immunological ageing, characterized by accumulation of senescent
(CD57⁺ or CD27/C28⁻) CD4⁺ and CD8⁺ T-cells, was not significantly different in HIV+ compared to HIV- COBRA participants, but was significantly higher when compared to BBD. Multivariate analysis showed that the increased levels of senescent T cells were strongly associated with CMV (co-)infection, but not HBV or HCV infection. In addition, CMV IgG levels which are considered a measure for CMV reactivation, were associated with increased T cell senescence.

**Conclusion:** Although CD4⁺ and CD8⁺ counts in the HIV+ group on suppressive cART were not restored to levels observed in uninfected controls, and CD4 T-cell activation and exhaustion was elevated, no evidence was found for increased immunological ageing when compared to appropriately selected controls. Increased T cell senescence in COBRA participants may be largely attributed to high prevalence of CMV infection in COBRA HIV+ and HIV- as compared to BBD. Had only samples from BBD been used as controls one would have falsely concluded that immunological ageing was increased in the HIV+ group. This illustrates the crucial importance of using appropriate controls that are comparable regarding lifestyle and risk behaviour for these studies.
Long-term monitoring of the effects of HIV infection and treatment in humanized mice.

Background
HIV-positive persons, even those on effective combination antiretroviral therapy (cART) may be at increased risk of developing some age-associated non-communicable comorbidities, for example cardiovascular disease, cognitive impairment, chronic kidney disease, osteoporosis and non-AIDS associated malignancies. The underlying pathogenesis remains elusive but might include effects on ageing. COBRA, an EU-funded project, aims at better understanding the causative links between HIV infection, cART, ageing and the development of such comorbidities.

Methods
To address this question, we generated 4 cohorts of Human Immune System (HIS) NSG mice that were HIV infected and treated with cART or not. Mice were monitored for 6 months after infection for a range of biomarkers including both plasma HIV-RNA viral load and T-cell count, activation (CD38⁺HLA-DR⁺) and senescence (CD57⁺).

Results
We first developed and validated a reliable method to deliver antiretroviral drugs (ARV’s) to HIS mice over a prolonged period of time whilst ensuring minimal alterations of their metabolism. By mixing ARV’s in MediDrop, a liquid gel replacing drinking water, we were able to treat mice without handling-related stress or alteration of their drink intake. As expected, untreated infected mice showed sustained high viral load, CD4⁺ depletion and T-cell activation. In mice treated with a combination of abacavir, lamivudine and dolutegravir HIV-RNA dropped to undetectable as soon as 2 weeks after treatment initiation, which was sustained until the end of the 6-month experiment. CD4⁺ T cell counts increased and T-cell activation decreased to a level comparable to that observed in uninfected mice.

Conclusions
We have validated an in vivo HIS mouse model that recapitulates HIV infection and long-term cART treatment. This model will be used to further dissect any potential effects of either cART or HIV on additional biomarkers related to (bone) metabolism, brain inflammation, and ageing.
Origin and function of circulating miRNA in long-living Ames dwarf mice

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Studies of mice with growth hormone (GH) deficiency or resistance have shown that disruption of the GH axis promotes insulin sensitivity and is strongly associated with extended longevity and delayed aging. Ames dwarf (df/df) mice are GH, PRL and TSH deficient. The disruption of GH signaling causes significant reduction of body size, plasma IGF1 and insulin, and most importantly extended longevity. Ames dwarf mice are known to maintain healthy insulin sensitivity, improved glucose homeostasis and low inflammatory status as they age. Recent studies demonstrated that serum and/or tissue levels of specific miRNA significantly change with age. The ability of circulating miRNAs to act as signaling molecules and regulate a broad spectrum of cellular functions implicates them as key players in the aging process. Our recent analysis of circulating miRNA showed genotype-specific changes in the circulating levels of 21 miRNAs during aging [genotype-by-age interaction (GbA)]. Genotype-by-age miRNAs showed four distinct expression patterns and significant overtargeting of transcripts involved in age-related processes. Functional enrichment analysis of validated miRNA targets highlighted cellular processes such as tumor suppression, anti-inflammatory response, and modulation of Wnt, insulin, mTOR, and MAPK signaling pathways. There is evidence that these miRNAs circulating in the bloodstream can be taken up by cells and alter expression of targeted genes in different tissues and cells. Importantly, our studies indicated that some of these pro-longevity miRNA are produced and released to circulation by adipose derived stem cells (ADSC), indicating potential new mechanism by which stem cells can regulate the transcriptome and regenerate affected surrounding cells and tissues or maintain cellular health during aging.
Rapamycin modulates cell senescence and inflammation by different mechanisms.
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Several recent studies suggest that senescent cells contribute to age-related pathology and loss of function. Since some of the beneficial effects of rapamycin appear to be mediated by inhibition of cell senescence, we are testing the mechanisms involved. Nrf2 is involved in the adaptive response of cells to different stresses. Nrf2 activity decreases with age, and a failure in this response is known to induce cellular senescence, so we chose to test whether Nrf2 was involved in the inhibition of cell senescence mediated by rapamycin.

Our preliminary data showed that rapamycin increased the levels of Nrf2 in WT mouse fibroblasts, and this increase correlates with decreased levels of stress-induced premature senescence (SIPS), as measured by β-galactosidase staining (b-gal) and the molecular markers p16 and p21. Furthermore, Nrf2 KO cells showed a significantly increased level of β-galactosidase staining, p16 and p21 even before SIPS treatment, and all these markers were even higher after SIPS treatment. As was observed in WT cells, rapamycin treatment significantly decreased b-gal staining, p16 and p21 levels in Nrf2 KO cells, more so than in WT cells. In addition, rapamycin significantly decreased the senescence associated secretory phenotype (SASP) in both WT and Nrf2 KO cells, measured by the levels of proinflammatory cytokines using a mouse cytokine array.

These effects were further corroborated \textit{in vivo}, using the Nrf2 knock out mouse, where rapamycin treatment (4mg/kg every other day for 6 weeks) led to a decrease in pro-inflammatory cytokines in serum, and decreased b-gal staining in fat tissue comparable with the data obtained in cells; however the effect on p16 levels were more variable depending on the tissue used, i.e., the levels of p16 were not significantly decreased in fat tissue, however it was significantly reduced in the lungs and brain.

To identify whether similar mechanism are observed in other types of cellular senescence, we used human diploid lung fibroblast WI-38 cells to study replicative senescence and the role of Nrf2 in the anti-senescence effect mediated by rapamycin. So far, our studies have shown that the senescence-associated changes in Nrf2 and p16 (decrease and increase, respectively) were delayed by rapamycin treatment. Interestingly, knocking out Nrf2 by CRISPR/Cas9 in a “no senescence state” significantly increased the levels of p16 protein and, at difference to WT cells, rapamycin treatment could not reduce it. This data suggest that while activation of autophagy by rapamycin requires Nrf2, inhibition of the cell senescence pathways can occur via both Nrf2-dependent and –independent pathways.